

***** STN Columbus *****

FILE 'HOME' ENTERED AT 12:52:10 ON 23 JUN 2003

=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull

=> e siddiqi salman/au

E1 2 SIDDIQI SAEEDUL HASAN/AU

E2 1 SIDDIQI SAJID/AU

E3 8 --> SIDDIQI SALMAN/AU

E4 18 SIDDIQI SALMAN H/AU

E5 1 SIDDIQI SALMAN UL HAQ/AU

E6 3 SIDDIQI SAMEEN/AU

E7 4 SIDDIQI SARFRAZ/AU

E8 1 SIDDIQI SAROSH/AU

E9 1 SIDDIQI SHABANA Q/AU

E10 8 SIDDIQI SHADAB A/AU

E11 3 SIDDIQI SHAH N/AU

E12 1 SIDDIQI SHAHAB A/AU

=> s e3-e5 and mycobact?

L1 26 ("SIDDIQI SALMAN"/AU OR "SIDDIQI SALMAN H"/AU OR "SIDDIQI
SALMAN

UL HAQ"/AU) AND MYCOBACT?

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 20 DUP REM L1 (6 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 20 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 20 MEDLINE

AN 2002182825 MEDLINE

DN 21871388 PubMed ID: 11880414

TI New simple and rapid test for culture confirmation of

Mycobacterium tuberculosis complex: a multicenter study.

AU Hasegawa Naoki; Miura Takao; Ishii Koudou; Yamaguchi Kazuhiro; Lindner

Thomas H; Merritt Samuel; Matthews Janis D; ***Siddiqi Salman H***

CS Cardiopulmonary Division, Department of Internal Medicine, Keio

University, School of Medicine, Tokyo, Japan.. hasegawn@sc.itc.keio.ac.jp

SO JOURNAL OF CLINICAL MICROBIOLOGY, (2002 Mar) 40 (3) 908-12.

Journal code: 7505564. ISSN: 0095-1137.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200204

ED Entered STN: 20020403

Last Updated on STN: 20020419

Entered Medline: 20020418

AB ***Mycobacterial*** antigen MPB64 has been identified as a

Mycobacterium tuberculosis complex-specific secretory protein since 1984. Recently, a simple culture confirmation test for M. tuberculosis complex has been developed by using lateral flow immunochromatographic assay (ICA) to detect MPB64 with anti-MPB64 monoclonal antibody. The current multicenter study evaluated the performance of an ICA slide test for MPB64 antigen in the clinical setting. Primary positive cultures from clinical specimens, as well as stock cultures, were tested. Approximately 100 microl of positive liquid culture medium or suspension made from colonies on solid medium was placed into the test well of the plastic slide device, and the test was read after 15 min. No processing or instrumentation was required. A total of 304 ***mycobacterial*** isolates consisting of M. tuberculosis complex (171 isolates) and ***mycobacteria*** other than M. tuberculosis (MOTT) complex (133 isolates) belonging to 18 different species were tested. Growth in liquid media (***Mycobacteria*** Growth Indicator Tube [MGIT] and Radiometric 12B), as well as in solid (Lowenstein-Jensen and Middlebrook 7H10 agar) media, was evaluated. Results were compared with those obtained with nucleic acid-based and/or high-pressure liquid chromatography identification. All MOTT were found to be negative on the ICA slide with no cross-reaction. All M. tuberculosis and M. africanum cultures were found to be positive, whereas the results of M. bovis and M. bovis BCG cultures were variable since some of the BCG strains are known to lack MPB64 antigen production. The results did not change with prolonged storage of cultures. This low-tech rapid test with high sensitivity and specificity could provide an alternative to currently available identification methods, particularly for recently introduced nonradiometric liquid culture systems such as MGIT.

L2 ANSWER 2 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE

1

AN 1999:519084 BIOSIS

DN PREV199900519084

TI Multicenter laboratory validation of susceptibility testing of
Mycobacterium tuberculosis against classical second-line and newer
antimicrobial drugs by using the radiometric BACTEC 460 technique and the
proportion method with solid media.

AU Pfyffer, Gaby E. (1); Bonato, Donald A.; Ebrahimzadeh, Adeleh; Gross,
Wendy; Hotaling, Jacqueline; Kornblum, John; Laszlo, Adalbert; Roberts,
Glenn; Salfinger, Max; Wittwer, Franziska; ***Siddiqi, Salman***

CS (1) Department of Medical Microbiology, Swiss National Center for
Mycobacteria, University of Zurich, Gloriastrasse 30, 8028, Zurich
Switzerland

SO Journal of Clinical Microbiology, (Oct., 1999) Vol. 37, No. 10, pp.
3179-3186.

ISSN: 0095-1137.

DT Article

LA English

SL English

AB In a large multicenter study involving six major study sites in the United States, Canada, and Europe, the susceptibilities of 272

Mycobacterium tuberculosis strains to classical second-line antituberculosis (anti-TB) drugs (capreomycin, cycloserine, ethionamide, and kanamycin) and newer compounds (amikacin, clofazimine, ofloxacin, and rifabutin) were determined by the radiometric BACTEC 460 procedure and the conventional proportion method on Middlebrook 7H10 agar. Previously established critical concentrations for classical second-line anti-TB drugs were compared with several concentrations in liquid medium to establish equivalence. MICs of newer compounds determined in liquid medium were either the same or up to four times lower than those determined in agar medium. After establishing critical concentrations (breakpoints) in the extended testing of clinical isolates, we obtained an excellent overall correlation between the two systems, with no errors with amikacin, kanamycin, and ofloxacin and very few major or very major errors with the other drugs; however, for cycloserine, no breakpoint concentration could be recommended due to repeatedly inconsistent results by both methods. Based on these data we conclude that the BACTEC 460 procedure is a simple and rapid method requiring 4 to 8 days on average to generate accurate antimicrobial susceptibility testing (AST) results for eight anti-TB drugs other than those considered primary ones. These data not only fill a major gap of knowledge regarding the critical test concentrations of secondary anti-TB drugs but also provide a baseline for future evaluations of M. tuberculosis AST with the more recently developed, nonradiometric broth-based culture systems.

L2 ANSWER 3 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE

2

AN 2000:31207 BIOSIS

DN PREV200000031207

TI Drug tolerance in ***Mycobacterium*** tuberculosis.

AU Wallis, Robert S. (1); Patil, Shripad; Cheon, Seon-Hee; Edmonds, Kay;

Phillips, Manijeh; Perkins, Mark D.; Joloba, Moses; Namale, Alice;

Johnson, John L.; Teixeira, Lucileia; Dietze, Reynaldo; ***Siddiqi,***

*** Salman*** ; Mugerwa, Roy D.; Eisenach, Kathleen; Ellner, Jerrold J.

CS (1) Case Western Reserve University School of Medicine, BRB 1034, 2109
Adelbert Rd., Cleveland, OH, 44106-4984 USA

SO Antimicrobial Agents and Chemotherapy, (Nov., 1999) Vol. 43, No. 11, pp.
2600-2606.

ISSN: 0066-4804.

DT Article

LA English

SL English

AB Although ***Mycobacterium*** tuberculosis is eradicated rapidly during therapy in some patients with pulmonary tuberculosis, it can persist for many months in others. This study examined the relationship between ***mycobacterial*** drug tolerance (delayed killing in vitro), persistence, and relapse. It was performed with 39 fully drug-susceptible isolates from a prospective trial of standard short-course antituberculous therapy with sputum smear-positive, human immunodeficiency virus-uninfected subjects with pulmonary tuberculosis in Brazil and Uganda. The rate of killing in vitro was determined by monitoring the growth index (GI) in BACTEC 12B medium after addition of drug to established cultures and was measured as the number of days required for 99% sterilization. Drugs differed significantly in bactericidal activity, in the following order from greatest to least, rifampin > isoniazid-ethambutol > ethambutol ($P < 0.001$). Isolates from subjects who had relapses ($n = 2$) or in whom persistence was prolonged ($n = 1$) were significantly more tolerant of isoniazid-ethambutol and rifampin than isolates from other subjects ($P < 0.01$). More generally, the duration of persistence during therapy was predicted by strain tolerance to isoniazid and rifampin ($P = 0.012$ and 0.026 , respectively). Tolerance to isoniazid-ethambutol and tolerance to rifampin were highly correlated ($P < 0.001$). Tolerant isolates did not differ from others with respect to the MIC of isoniazid; the rate of killing of a tolerant isolate by isoniazid-ethambutol was not increased at higher drug concentrations. These observations suggest that tolerance may not be due to drug-specific mechanisms. Tolerance was of the phenotypic type, although increased tolerance appeared to emerge after prolonged drug exposure in vivo. This study suggests that drug tolerance may be an important determinant of the outcome of therapy for tuberculosis.

L2 ANSWER 4 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE

3

AN 1997:155949 BIOSIS

DN PREV199799455152

TI Characterization of pncA mutations in pyrazinamide-resistant
Mycobacterium tuberculosis.

AU Scorpio, Angelo; Lindholm-Levy, Pamela; Heifets, Leonid; Gilman, Robert;
Siddiqi, Salman ; Cynamon, Michael; Zhang, Ying (1)

CS (1) Dep. Mol. Microbiol. Immunol., Sch. Hygiene Public Health, Johns
Hopkins Univ., 615 N. Wolfe St., Baltimore, MD 21205 USA

SO Antimicrobial Agents and Chemotherapy, (1997) Vol. 41, No. 3, pp. 540-543.
ISSN: 0066-4804.

DT Article

LA English

AB Pyrazinamide (PZA) is a first-line drug for short-course tuberculosis therapy. Resistance to PZA is usually accompanied by loss of pyrazinamidase (PZase) activity in ***Mycobacterium*** tuberculosis. PZase converts PZA to bactericidal pyrazinoic acid, and the loss of PZase activity is associated with PZA resistance. The gene (pncA) encoding the M. tuberculosis PZase has recently been sequenced, and mutations in pncA were previously found in a small number of PZA-resistant M. tuberculosis strains. To further understand the genetic basis of PZA resistance and determine the frequency of PZA-resistant strains having pncA mutations, we analyzed a panel of PZA-resistant clinical isolates and mutants made in vitro. Thirty-three of 38 PZA-resistant clinical isolates had pncA mutations. Among the five strains that did not contain pncA mutations, four were found to be falsely resistant and one was found to be borderline resistant to PZA. The 33 PZA-resistant clinical isolates and 8 mutants made in vitro contained various mutations, including nucleotide substitutions, insertions, or deletions in the pncA gene. The identified mutations were dispersed along the pncA gene, but some degree of clustering of mutations was found at the following regions: Gly132-Thr142, Pro69-Leu85, and Ile5-Asp12. PCR-single-strand conformation polymorphism (SSCP) analysis was shown to be useful for the rapid detection of pncA mutations in the PZA-resistant strains. We conclude that a mutation in the pncA gene is a major mechanism of PZA resistance and that direct sequencing by PCR or SSCP analysis should help to rapidly identify PZA-resistant M. tuberculosis strains.

L2 ANSWER 5 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1996:134250 BIOSIS

DN PREV199698706385

TI Radiomicrobiology.

AU ***Siddiqi, Salman H.***

CS Becton Dickinson Diagnostic Instruments Systems, Sparks, MD USA

SO Harbert, J. C. [Editor]; Eckelman, W. C. [Editor]; Neumann, R. D.

[Editor]. (1996) pp. 331-342. Nuclear medicine: Diagnosis and therapy.

Publisher: Thieme Medical Publishers, Inc. Suite 1501, 381 Park Avenue South, New York, New York 10016, USA.

ISBN: 0-86577-570-2, 3-13-101121-1.

DT Book

LA English

L2 ANSWER 6 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

4

AN 1996:78048 BIOSIS

DN PREV199698650183

TI Growth inhibition of ***Mycobacterium*** tuberculosis by polyoxyethylene stearate present in the BACTEC pyrazinamide susceptibility test.

AU Miller, Mark A. (1); Thibert, Louise; Desjardins, F; ***Siddiqi, Salman***
*** H.*** ; Dascal, A.

CS (1) McGill Univ., SMBD-Jewish Gen. Hosp., Montreal, PQ Canada

SO Journal of Clinical Microbiology, (1996) Vol. 34, No. 1, pp. 84-86.

ISSN: 0095-1137.

DT Article

LA English

AB We have previously found that approximately 3.5% of 428 clinical isolates of ***Mycobacterium*** tuberculosis yield uninterpretable results in the BACTEC pyrazinamide (PZA) susceptibility test system, because of inadequate growth. We tested the hypothesis that polyoxyethylene stearate (POES), the ingredient of the reconstituting fluid for the test, was the cause of this growth inhibition. A total of 15 isolates known for their previously uninterpretable results and 100 randomly chosen clinical isolates were tested in parallel both with and without POES. Repeat testing of the isolates with previously uninterpretable results yielded results in the presence of POES in only seven (47%). In the absence of POES, all gave interpretable results but one such result showed false resistance. For the other 100 clinical isolates, interpretable results were obtained with and without POES, but growth was enhanced in the absence of POES, especially in the PZA-susceptible strains. This was evidenced by a decreased time to attain a growth index of 200 in the control vial) (4.9 days without POES versus 5.8 days with POES; P lt 0.001) and a higher mean growth index ratio on the day of interpretation of the test (7.4% without POES versus 2.2% with POES; P lt 0.001). However, the enhanced growth without POES led to 20 susceptible strains being misinterpreted as either resistant or borderline. We suggest that isolates of M. tuberculosis which yield uninterpretable results in the BACTEC PZA test system should be retested both with and without POES. If interpretable results indicating PZA resistance are obtained only in the absence of POES, the result should be confirmed by a pyrazinamidase assay or by the conventional proportion method. Routine omission of POES from the BACTEC test for all clinical strains is discouraged because of the unacceptably high false-resistance rates.

L2 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1995:810849 CAPLUS

DN 123:193058

TI Assays for ***Mycobacterium*** tuberculosis using monospecific antibodies

IN ***Siddiqi, Salman H.*** ; Rahman, Raisur A. F.; Root, Richard Terry

PA Rodrick, Richard J., USA

SO PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI WO 9519781	A1	19950727	WO 1994-US14685	19941220
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W: AU, BR, CA, CN, JP, KR

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

AU 9515534	A1	19950808	AU 1995-15534	19941220
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PRAI US 1994-187235 19940128

WO 1994-US14685 19941220

AB Monospecific antibodies which selectively bind to ***Mycobacterium*** tuberculosis are described. The monospecific antibodies include monoclonal antibodies and monospecific polyclonal antibodies. Methods of detecting the presence of ***Mycobacterium*** tuberculosis using monospecific antibodies are described. The assay methods include the use of flow-through immunoassay test devices.

L2 ANSWER 8 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE

5

AN 1995:439407 BIOSIS

DN PREV199598453707

TI Testing of susceptibility of ***Mycobacterium*** tuberculosis to pyrazinamide: Comparison of bactec method with pyrazinamidase assay.

AU Miller, Mark A. (1); Thibert, Louise; Desjardins, F.; ***Siddiqi, Salman***
*** H.*** ; Dascal, A.

CS (1) McGill Univ., Dep Microbiol., SMBD-Jewish General Hosp., Montreal
Canada

SO Journal of Clinical Microbiology, (1995) Vol. 33, No. 9, pp. 2468-2470.

ISSN: 0095-1137.

DT Article

LA English

AB The susceptibility of 428 clinical isolates of ***Mycobacterium*** tuberculosis to pyrazinamide was assessed by the Bactec method and the Wayne pyrazinamidase assay. The correlation between the two tests was 98.2 and 100% for susceptible and resistant strains, respectively. False resistance was seen in four (0.8%) strains with the Bactec test, and false-susceptible results occurred in two (0.5%) pyrazinamidase assays. The Bactec test is rapid and reliable, and the Bactec results correlate well with the pyrazinamidase test results, although some strains did not grow well in the test medium.

L2 ANSWER 9 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1995:290163 BIOSIS

DN PREV199598304463

TI Growth inhibition of ***Mycobacterium*** tuberculosis and
Mycobacterium avium by plant compounds and extracts.

AU Sader, Camil; ***Siddiqi, Salman*** ; Nhung The Hang; Norris, Leslie;
To, Leleng

CS Dep. Biol. Sci., Goucher Coll., Towson, MD 21204 USA

SO Abstracts of the General Meeting of the American Society for Microbiology,
(1995) Vol. 95, No. 0, pp. 146.

Meeting Info.: 95th General Meeting of the American Society for
Microbiology Washington, D.C., USA May 21-25, 1995

ISSN: 1060-2011.

DT Conference

LA English

L2 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1993:555520 CAPLUS

DN 119:155520

TI Disinfectants and lysing agents in protocol for release of intracellular
components

IN Dey, Margaret Sigler; Keating, William Edward; ***Siddiqi, Salman ul***
*** Haq*** ; Down, James Arthur

PA Becton Dickinson and Co., USA

SO Eur. Pat. Appl., 23 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI EP 552571	A1	19930728	EP 1992-311874	19921231
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R: DE, ES, FR, GB, IT, SE

CA 2086729	AA	19930710	CA 1993-2086729	19930105
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AU 9331113	A1	19930715	AU 1993-31113	19930108
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JP 05317033	A2	19931203	JP 1993-2851	19930111
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PRAI US 1992-819355 19920109

AB A sample processing protocol comprises (a) adding a disinfectant to a sample, and (b) lysing microorganisms present in (a). The protocol is rapid, simple, and provides intracellular components capable of detection, amplification, and the like. In addn., the sample is rendered safe for handling. The lysing reagent esp. comprises proteinase K, achromopeptidase, and EDTA. The sample is liquefied prior to step (a) with a soln. comprising NaOH, Na citrate, and N-acetyl-L-cysteine.

Optimization and use of the protocol to amplify and detect
Mycobacterium tuberculosis DNA by PCR are described.

L2 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1994:27035 CAPLUS

DN 120:27035

TI Microbial sample processing using a disinfectant for lysis

IN Dey, Margaret S.; Keating, William E.; ***Siddiqi, Salman H.*** ; Down,
James A.

PA Becton, Dickinson and Co., USA

SO Can. Pat. Appl., 38 pp.

CODEN: CPXXEB

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI CA 2086725	AA	19930710	CA 1993-2086725	19930105
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EP 556521	A1	19930825	EP 1992-311875	19921231
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R: DE, ES, FR, GB, IT, SE

AU 9331109	A1	19930805	AU 1993-31109	19930108
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JP 05276929	A2	19931026	JP 1993-2849	19930111
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PRAI US 1992-819353 19920109

AB Disinfectants (O-Syl, Roccal II, Vesphene, Chlorox, Staphene, Lysol,

Hisbiclens) are used for the lysis of microorganisms such as

mycobacteria . The method, which is simple, rapid and safe, may be
used, i.a., for the isolation of nucleic acids. Thus, 1% Staphene extd.
amplifiable amts. of DNA from M. tuberculosis.

L2 ANSWER 12 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC.DUPLICATE

6

AN 1993:430052 BIOSIS

DN PREV199396084677

TI Rapid broth macrodilution method for determination of MICs for

Mycobacterium avium isolates.

AU ***Siddiqi, Salman H. (1)*** ; Heifets, Leonid B.; Cynamon, Michael H.;

Hooper, Nancy M.; Laszlo, Adalbert; Libonati, Joseph P.; Lindholm-Levy,

Pamela J.; Pearson, Nicole

CS (1) Res. Dev., Becton Dickinson Diagnostic Instrument Systems, 7 Loveton
Circle, Sparks, MD 21152 USA

SO Journal of Clinical Microbiology, (1993) Vol. 31, No. 9, pp. 2332-2338.

ISSN: 0095-1137.

DT Article

LA English

AB A multicenter study was done to investigate the accuracy and reproducibility of a method for determining the MICs of antimicrobial agents against the ***Mycobacterium*** avium complex in 7HI2 broth with the BACTEC system. In phase I, with eight drugs and 10 strains, intralaboratory reproducibility was 95.7 to 100%, allowing a 1-dilution difference upon repeat testing. The results of phase II testing with 41 additional strains were consistent with those obtained in phase I, with good interlaboratory reproducibility. The radiometric method was validated by sampling and plating of the same broth cultures and determining, by the number of CFU per milliliter, the lowest drug concentration that inhibited more than 99% of the initial bacterial population. Three test concentrations of each drug and the tentative interpretation of results are proposed. Radiometric MIC determination has the potential to become the method of choice for clinical microbiology laboratories and evaluation of new agents for the treatment of M. avium infections, both pulmonary and disseminated.

L2 ANSWER 13 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1994:128775 BIOSIS

DN PREV199497141775

TI ***Mycobacteriology*** : Past, present and future.

AU ***Siddiqi, Salman H.***

SO Igiene Moderna, (1993) Vol. 99, No. 5, pp. 629-631.

ISSN: 0019-1655.

DT Article

LA English

L2 ANSWER 14 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AN 1993:299781 BIOSIS

DN PREV199396018006

TI Bacteriologic investigations of unusual ***mycobacteria*** isolated from immunocompromised patients.

AU ***Siddiqi, Salman H. (1)*** ; Laszlo, Adalbert; Butler, W. Ray; Kilburn, James O.

CS (1) Becton Dickinson Diagnostic Instrument Systems, R and D Dep., 7 Loveton Circle, Sparks, MD 21152 USA

SO Diagnostic Microbiology and Infectious Disease, (1993) Vol. 16, No. 4, pp. 321-323.

ISSN: 0732-8893.

DT Article

LA English

AB ***Mycobacterial*** isolates from blood and other extrapulmonary sites of six patients with AIDS were investigated because the isolates grew only in liquid media and failed to grow on solid culture media even on subculturing. Our investigations indicated that these ***mycobacteria***

possess common, but unusual, characteristics and probably belong to an unrecognized species recently reported as " ***Mycobacterium*** genavense."

L2 ANSWER 15 OF 20 USPATFULL

AN 88:57253 USPATFULL

TI Method and composition for enhancement of growth of ***mycobacteria***

IN ***Siddiqi, Salman H.***, Timonium, MD, United States

Broman, Rodney L., Fallston, MD, United States

PA Becton Dickinson and Company, Franklin Lakes, NJ, United States (U.S. corporation)

PI US 4769332 19880906

AI US 1985-773740 19850906 (6)

RLI Continuation-in-part of Ser. No. US 1983-548476, filed on 3 Nov 1983, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Rosen, Sam

LREP McBride, James R.

CLMN Number of Claims: 11

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 209

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A growth medium for ***mycobacteria*** is provided which has enhanced growth properties. The growth medium contains an effective amount of polyoxyethylene stearate and an albumin.

L2 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1988:587123 CAPLUS

DN 109:187123

TI Direct antimicrobial drug susceptibility testing of ***Mycobacterium*** tuberculosis by the radiometric method

AU Libonati, Joseph P.; Stager, Charles E.; Davis, James R.; ***Siddiqi,***
*** Salman H.***

CS Maryland Dep. Health Ment. Hyg., Baltimore, MD, USA

SO Diagnostic Microbiology and Infectious Disease (1988), 10(1), 41-8
CODEN: DMIDDZ; ISSN: 0732-8893

DT Journal

LA English

AB Direct-drug-susceptibility tests were performed on clin. specimens pos. for acid-fast bacilli by either Ziehl-Neelsen or fluorochrome staining. The results of conventional agar diln. (Vestal, 1975) and a modified radiometric (BACTEC) method were compared. A total of 580 smear-pos. specimens were tested by the BACTEC method at three sep. sites. Of these

377 were culture pos. for *M. tuberculosis*, and 343 (91%) yielded acceptable direct-susceptibility-test results. The conventional method showed that 343 of 519 smear-pos. specimens were culture pos. for *M. tuberculosis*, and 212 (62%) produced acceptable results within 3 wks. Conventional results were reported in 3-4 wks, while the time required to obtain results with the BACTEC method ranged from 5 to 21 days (av. 11.5 days). Results indicate that the radiometric method provides reportable results more frequently with time savings as compared to the conventional method.

L2 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1986:65719 CAPLUS

DN 104:65719

TI Interlaboratory drug susceptibility testing of ****Mycobacterium**** tuberculosis by a radiometric procedure and two conventional methods

AU ***Siddiqi, Salman H.*** ; Hawkins, Jean E.; Laszlo, Adalbert

CS Johnston Lab., Towson, MD, 21204, USA

SO Journal of Clinical Microbiology (1985), 22(6), 919-23

CODEN: JCMIDW; ISSN: 0095-1137

DT Journal

LA English

AB A total of 224 recent isolates of *M. tuberculosis* from 163 patients were tested for multidrug resistance against streptomycin (SM), isoniazid, rifampin, and ethambutol (EMB) by the rapid radiometric BACTEC method and 2 conventional proportion methods: the World Health Organization (WHO) method, using Lowenstein-Jensen medium and the Veterans Administration ref. lab. for ***mycobacteria*** (VA) method, using Middlebrook 7H10 agar medium. The results were compared, focusing on the concns. of the drugs in all 3 methods. Among the 4 drugs tested, most of the discrepancies in measured activity were obsd. with SM and EMB, generally because of differences in the drug concns. used by the 3 methods. A 4-.mu.g amt. of SM in the BACTEC method was slightly less active than 10 .mu.g in the VA method and significantly more active than 4 .mu.g of dihydrostreptomycin in the WHO method. With EMB, 2.5 .mu.g in BACTEC was similar to 5 .mu.g in the VA method and 2 .mu.g in the WHO method, while 10 .mu.g in the BACTEC method was more active than 10 and 2 .mu.g in the VA and WHO methods, resp. To attain close agreement, drug concns. used in the BACTEC method should be carefully selected when a comparison is to be made with any conventional method employed in a lab.

L2 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1984:451153 CAPLUS

DN 101:51153

TI Evaluation of a rapid radiometric differentiation test for the ****Mycobacterium**** tuberculosis complex by selective inhibition with

p-nitro-.alpha.-acetylamino-.beta.-hydroxypropiophenone
AU Laszlo, Adalbert; ***Siddiqi, Salman H.***
CS Natl. Reference Cent. Tuberculosis, Health and Welfare Canada, Ottawa, ON,
K1A 0L2, Can.
SO Journal of Clinical Microbiology (1984), 19(5), 694-8
CODEN: JCMIDW; ISSN: 0095-1137
DT Journal
LA English
AB A rapid technique for the differentiation of the M. tuberculosis complex
from other ***mycobacteria*** was evaluated with p-nitro-.alpha.-
acetylamino-.beta.-hydroxypropiophenone (NAP) as a selective inhibitory
agent. Some 416 coded cultures, 234 cultures belonging to the M.
tuberculosis complex and 182 cultures belonging to 35 other
mycobacterial species, were tested in 2 labs. for NAP inhibition
with 5 and 10 .mu.g NAP/mL in Middlebrook 7H12 liq. medium. The indirect
testing mode, in which a large bacterial inoculum was used from an
isolated culture on a solid medium, and the direct testing mode, which
used a small inoculum from 7H12 medium, were compared. A decrease or no
increase in daily 14CO₂ output as measured by a BACTEC system was
considered evidence of inhibition. Apparently, 5 .mu.g NAP/mL can
effectively sep. the M. tuberculosis complex from other
mycobacterial species in 4-6 days. The direct test data show
that, unlike other conventional biochem. tests, it does not require a
heavy inoculum of ***mycobacteria*** and can therefore be performed
soon after growth is detected by the radiometric method.

L2 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1985:20838 CAPLUS

DN 102:20838

TI Rapid radiometric methods to detect and differentiate
Mycobacterium tuberculosis/M. bovis from other
mycobacterial species

AU ***Siddiqi, Salman H.*** ; Hwangbo, Charles C.; Silcox, Vella; Good,
Robert, C.; Snider, Dixie E., Jr.; Middlebrook, Gardner

CS Johnston Lab., Towson, MD, USA

SO American Review of Respiratory Disease (1984), 130(4), 634-40

CODEN: ARDSBL; ISSN: 0003-0805

DT Journal

LA English

AB Rapid methods for the differentiation of M. tuberculosis/M. bovis (TB
complex) from other ***mycobacteria*** (MOTT bacilli) were developed
and evaluated in a 3-phase study. In the 1st phase, techniques for
identification of ***Mycobacterium*** species were developed by using
radiometric technol. and BACTEC Middlebrook 7H12 liq. medium. Based on
14CO₂ evolution, characteristic growth patterns were established for 13

commonly encountered ***mycobacterial*** species.

Mycobacteria belonging to the TB complex were differentiated from other ***mycobacteria*** by cellular morphol. and rate of $^{14}\text{CO}_2$ evolution. For further differentiation, radiometric tests for niacin prodn. and inhibition by o-nitro-.alpha.-acetyl amino-.beta.-hydroxypropio-phenone (NAP) were developed. In the 2nd phase, 100 coded specimens on Lowenstein-Jensen medium were identified as members of the TB complex, MOTT bacilli, bacteria other than ***mycobacteria***, or no viable organisms within 3-12 (av. 6.4) days of receipt from the Centers for Disease Control. Isolation and identification of ***mycobacteria*** from 20 simulated sputum specimens were carried out in Phase III. Out of 20 sputum specimens, 16 contained culturable ***mycobacteria***, and all of the positives were detected by the BACTEC method in an av. of 7.3 days. The pos. ***mycobacterial*** cultures were isolated and identified as TB complex or MOTT bacilli in an av. of 12.8 days. The radiometric NAP test was highly sensitive and specific for a rapid identification of TB complex, whereas the radiometric niacin test had some inherent problems. Radiometric BACTEC and conventional methodologies were in complete agreement in Phase II as well as in Phase III.

L2 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2003 ACS

AN 1981:401115 CAPLUS

DN 95:1115

TI Evaluation of a rapid radiometric method for drug susceptibility testing of ***Mycobacterium*** tuberculosis

AU ***Siddiqi, Salman H.***; Libonati, Joseph P.; Middlebrook, Gardner

CS Sch. Med., Univ. Maryland, Baltimore, MD, 21201, USA

SO Journal of Clinical Microbiology (1981), 13(5), 908-12

CODEN: JCMIDW; ISSN: 0095-1137

DT Journal

LA English

AB A total of 106 isolates of *M. tuberculosis* were tested for drug susceptibility by the conventional 7H11 plate method and by a new rapid radiometric method using special 7H12 liq. medium with ^{14}C -labeled substrate. Results obtained by the 2 methods were compared for rapidity, sensitivity, and specificity of the new test method. There was 98% overall agreement between the results obtained by the 2 methods. Of a total of 424 drug tests, only 8 drug results did not agree, mostly in the case of streptomycin [57-92-1]. This new procedure was rapid, with 87% of the test results reportable within 4 days and 98% reportable within 5 days as compared with the usual 3 wks required with the conventional indirect susceptibility test method. The rapid radiometric method seems to have the potential for routine lab. use and merits further investigations.

=> file uspatfull

FILE 'USPATFULL' ENTERED AT 12:54:10 ON 23 JUN 2003

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 19 Jun 2003 (20030619/PD)

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HIGHEST GRANTED PATENT NUMBER: US6581208

HIGHEST APPLICATION PUBLICATION NUMBER: US2003115652

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ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 19 Jun 2003 (20030619/PD)

REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2003

USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2003

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This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s antibod?/clm and mycobact?/clm and antigen?/clm

23689 ANTIBOD?/CLM

1094 MYCOBACT?/CLM

12809 ANTIGEN?/CLM

L3 104 ANTIBOD?/CLM AND MYCOBACT?/CLM AND ANTIGEN?/CLM

=> s l3 and (complex?/clm)

48618 COMPLEX?/CLM

L4 40 L3 AND (COMPLEX?/CLM)

=> s (mpb64 or mpt64) and l4

18 MPB64

21 MPT64

L5 4 (MPB64 OR MPT64) AND L4

=> d bib ab clm 1-

YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 1 OF 4 USPATFULL

AN 2003:13073 USPATFULL

TI Early detection of mycobacterial disease

IN Laal, Suman, Croton-on-Hudson, NY, United States

Zolla-Pazner, Susan, New York, NY, United States

Belisle, John T., Fort Collins, CO, United States

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Colorado State University Research Foundation, Fort Collins, CO, United States (U.S. corporation)

PI US 6506384 B1 20030114

AI US 1999-396347 19990914 (9)

RLI Continuation-in-part of Ser. No. US 1997-1984, filed on 31 Dec 1997, now patented, Pat. No. US 6245331

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P

LREP Livnat, Shmeul, Venable, Baetjer, Howard & Civiletti

CLMN Number of Claims: 40

ECL Exemplary Claim: 1

DRWN 33 Drawing Figure(s); 39 Drawing Page(s)

LN.CNT 5685

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A number of protein and glycoprotein antigens secreted by Mycobacterium tuberculosis (Mt) have been identified as "early" Mt antigens on the basis early antibodies present in subjects infected with Mt prior to the development of detectable clinical disease. These early Mt antigens, in particular an 88 kDa secreted protein having a pI of about 5.2 and the sequence of SEQ ID NO:106, which is present in Mt lipoarabinomannan-free culture filtrate, a protein characterized as Mt antigen 85C; a protein characterized as Mt antigen MPT51, a glycoprotein characterized as Mt antigen MPT32; and a 49 kDa protein having a pI of about 5.1, are useful in immunoassay methods for early, rapid detection of TB in a subject. Preferred immunoassays detect the antibodies in the subject's urine. Also provided are antigenic compositions, kits and methods to useful for detecting an early Mt antigen, an early Mt antibody, and immune complexes thereof. For the first time, a surrogate marker is available for inexpensive screening of individuals at heightened risk for

developing advanced TB, in particular HIV-1 infected subjects and other immunocompromised individuals.

CLM What is claimed is:

1. A method for the early detection of active ***mycobacterial*** disease or infection in a subject, comprising assaying a biological fluid sample from a subject having symptoms of active tuberculosis, but before the onset of symptoms identifiable as advanced tuberculosis that is distinguished by (a) smear positivity of sputum for acid fast bacilli, (b) cavitory pulmonary lesions, or both (a) and (b), for the presence of early ***antibodies*** specific for a 27 kDa M. tuberculosis protein named MPT51 having the following properties: (i) is present in M. tuberculosis culture filtrate; (ii) has a pI of about 5.91; (iii) is reactive with a monoclonal ***antibody*** designated IT-52; (iv) is reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear-positivity of sputum for acid-fast bacilli and cavitory pulmonary lesions; and (v) is non reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, wherein the presence of said early ***antibodies*** specific for said protein is indicative of the presence of said disease or infection.

2. A method for the early detection of active ***mycobacterial*** disease or infection in a subject, comprising assaying a biological fluid sample: from a subject having symptoms of active tuberculosis, but before the onset of symptoms identifiable as advanced tuberculosis that is distinguished by (a) smear positivity of sputum for acid fast bacilli, (b) cavitory pulmonary lesions, or both (a) and (b), for the presence of immune ***complexes*** consisting of a 27 kDa M. tuberculosis protein named MPT51 having the following properties: (i) is present in M. tuberculosis culture filtrate; (ii) has a pI of about 5.91; (iii) is reactive with a monoclonal ***antibody*** designated IT-52; (iv) is reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear-positivity of sputum for acid-fast bacilli and cavitory pulmonary lesions; and (v) is non reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, wherein the presence of said immune ***complexes*** is indicative of the presence of said disease or infection.

3. The method of claim 1, which includes assaying said sample for ***antibodies*** specific for one or more additional early ***antigens*** of M. tuberculosis selected from the group consisting of: (a) an 88 kDa M. tuberculosis protein having the following properties: (1) present in M. tuberculosis culture filtrate; (2) pI of about 5.2; (3) an amino acid sequence SEQ ID NO:106;

MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KVVADLTPQN
QALLNARDEL

QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT
TTAGPQLVVP

VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAKEGPTY NKVRGDKVIA
YARKFLDDSV

PLSSGSFGDA TGFTVQDGQL VVALPKSTG LANPGQFAGY TGAAESPTSV
LLINHGLHIE

ILDPESQVG TTDRAKVVDV ILESAITTIM DFEDSVAADV AADKVLGYRN
WLGLNKGDLA

AAVDKDGTAFLRVLNDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTNDAL
VDTDGSEVFE

GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC
ELFSRVEDVL

GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRVVFINTGF LDRTGDEIHT
SMEAGPMVRK

GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI
AQPRAGASTA

WVPSPTAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA
PDEIREEVDN

NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH
GVITSADVRA

SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT
EPILHRRRRE

FKARAAEKPA PSDRAGDDAA R (b) a protein characterized as M. tuberculosis

antigen 85C; and (c) a glycoprotein characterized as M.

tuberculosis ***antigen*** MPT32.

4. The method of claim 1, comprising, prior to said assaying step, the
step of removing from said sample ***antibodies*** specific for
cross-reactive epitopes or ***antigens*** of proteins present in M.
tuberculosis and in other bacterial genera.

5. A method according to claim 4, wherein said removing is performed by immunoadsorption of said sample with E. coli ***antigens*** .

6. A method according to claim 1, wherein said subject is a human.

7. A method according to claim 6, wherein said subject is infected with HIV-1 or is at high risk for tuberculosis.

8. The method of claim 1, 3 or 2, comprising, before said assaying step, the step of obtaining said biological fluid sample from said subject.

9. The method of claim 4, comprising, before said assaying step, the step of obtaining said biological fluid sample from said subject.

10. The method of claim 6 comprising, before said assaying step, the step of obtaining said biological fluid sample from said subject.

11. The method of claim 7 comprising, before said assaying step, the step of obtaining said biological fluid sample from said subject.

12. The method of claim 1 or 3, wherein said biological fluid is urine.

13. The method of claim 4, wherein said biological fluid is urine.

14. The method of claim 5, wherein said biological fluid is urine.

15. The method of claim 6, wherein said biological fluid is urine.

16. The method of claim 7, wherein said biological fluid is urine.

17. The method of claim 2, wherein said biological fluid is urine.

18. The method of claim 8, wherein said biological fluid is urine.

19. The method of claim 9, wherein said biological fluid is urine.

20. The method of claim 1, 3 or 2 which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

21. The method of claim 4, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

22. The method of claim 5, which further includes a test that detects

mycobacterial bacilli in a sample of sputum or other body fluid of said subject.

23. The method of claim 6 which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

24. The method of claim 7, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

25. The method of claim 8, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

26. The method of claim 9, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

27. The method of claim 12, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

28. The method of claim 13, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

29. The method of claim 14, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

30. The method of claim 18, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

31. The method of claim 19, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

32. A kit useful for early detection of M. tuberculosis disease comprising: (a) an ***antigenic*** composition comprising a 27 kDa M. tuberculosis protein named MPT51 which is present in M. tuberculosis culture filtrate, has a pI of about 5.91, is reactive with a monoclonal ***antibody*** designated IT-52, and which is further characterized as being (i) reactive with ***antibodies*** found in tuberculosis

patients who are in a stage of disease prior to the onset of (1) smear positivity of sputum for acid fast bacilli, (2) cavitary pulmonary lesions, or both (1) and (2), and (ii) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, in combination with (b) reagents necessary for detection of ***antibodies*** which bind to said M. tuberculosis protein.

33. A kit useful for early detection of M. tuberculosis disease comprising: (a) an ***antigenic*** composition consisting essentially of two or more early M. tuberculosis ***antigens*** each of which is characterized as being (i) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of (1) smear positivity of sputum for acid fast bacilli, (2) cavitary pulmonary lesions, or both (1) and (2), and (ii) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, which ***antigenic*** composition includes a 27 kDa M. tuberculosis protein named MPT51 present in M. tuberculosis culture filtrate which has a pI of about 5.91, and is reactive with a monoclonal ***antibody*** designated IT-52; in combination with (b) reagents necessary for detection of ***antibodies*** which bind to said early M. tuberculosis ***antigens***.

34. A kit according to claim 32 or 33 wherein at least one of said early M. tuberculosis ***antigens*** is a recombinant protein or glycoprotein.

35. A kit according to claim 32 or 33, which further comprises at least one monoclonal ***antibody*** specific for an epitope of said early M. tuberculosis ***antigens***.

36. A kit useful for early detection of M. tuberculosis disease, comprising: (a) an ***antigenic*** composition that includes: (i) an isolated 27 kDa M. tuberculosis protein named MPT51 having the following properties: (1) present in M. tuberculosis culture filtrate; (2) pI of about 5.91; (3) reactive with a monoclonal ***antibody*** designated IT-52; (4) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear-positivity of sputum for acid-fast bacilli and cavitary pulmonary lesions; and (5) non reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis (ii) supplemented with one or more isolated early M. tuberculosis ***antigenic*** proteins of a second set, characterized as in (5) and (6) above, obtainable from 14 day cultures of M. tuberculosis strain H37Rv grown in glycerol alanine salts medium, and selected from the group consisting of: (1) a 28 kDa protein having a pI of about 5.1, corresponding to the

spot identified as Ref No. 77 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; (2) a 29/30 kDa protein having a pI of about 5.1, and corresponding to a spot identified as Ref No. 69 or 59 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; (3) a 31 kDa protein having a pI of about 5.1 and an N-terminal amino acid sequence FSRPGLPVEYLQVPSP (SEQ ID NO:95), and corresponding to a spot identified as Ref No. 103 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; (4) a 35 kDa protein having a pI of about 5.1 and an N-terminal amino acid sequence CGSKPPSPET (SEQ ID NO:87), and corresponding to a spot identified as Ref. No. 66 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11 and reacting with monoclonal ***antibody*** IT-23; (5) a 42 kDa protein having a pI of about 5.1, and corresponding to a spot identified as Ref. No. 68 or 80 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; (6) a 48 kDa protein having a pI of about 4.8, and corresponding to a spot identified as Ref. No. 24 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; and (7) a 104 kDa protein having a pI of about 5.1, and corresponding to a spot identified as Ref. No. 111 in FIGS. 15A-F, FIG. 18, Table 9 or Table 11; which spots are obtained by 2-dimensional electrophoretic separation of M. tuberculosis lipoarabinomannan-free culture filtrate proteins as follows: (A) incubating 3 hours at 20.degree. C. in 9M urea, 2% Nonidet P-40, 5% .beta.-mercaptoethanol, and 5% ampholytes at pH 3-10; (B) isoelectric focusing on 6% polyacrylamide isoelectric focusing tube gel of 1.5 mm.times.6.5 cm, said gel containing 5% ampholytes in a 1:4 ratio of pH 3-10 ampholytes to pH 4-6.5 ampholytes for 3 hours at 1 kV using 10 mM H.sub.3PO.sub.4 as catholyte and 20 mM NaOH as anolyte, to obtain a focused gel; (C) subjecting the focused gel to SDS PAGE in the second dimension by placement on a preparative SDS-polyacrylamide gel of 7.5.times.10 cm.times.1.5 mm containing a 6% stack over a 15% resolving gel and electrophoresing at 20 mA per gel for 0.3 hours followed by 30 mA per gel for 1.8 hours; said ***antigenic*** composition in combination with (b) reagents necessary for detection of ***antibodies*** which bind to said early M. tuberculosis ***antigens***.

37. The kit of claim 35 wherein said ***antigen*** of said second set is the 29/30 kDa protein.

38. The kit of any of claims 32, 33, 36, 37, that includes an early ***antigen*** selected from the group consisting of: (a) an 88 kDa M. tuberculosis protein having the following properties: (1) present in M. tuberculosis culture filtrate; (2) pI of about 5.2; (3) an amino acid sequence SEQ ID NO:106:

MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KVVADLTPQN
QALLNARDEL

QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT
TTAGPQLVVP

VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAEKGPTY NKVRGDKVIA
YARKFLDDSV

PLSSGSFGDA TGFTVQDGQL VVALPKSTG LANPGQFAGY TGAAESPTSV
LLINHGLHIE

ILIDPESQVG TTDRAQVKDV ILESATTIM DFEDSVAADV AADKVLGYRN
WLGLNKGDLA

AAVDKDGTAFLRVLNDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTNDAL
VDTDGSEVFE

GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC
ELFSRVEDVL

GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRVVFINTGF LDRTGDEIHT
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GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI
AQPRAGASTA

WVPSPTAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA
PDEIREEDVN

NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH
GVITSADVRA

SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT
EPILHRRRRE

FKARAAEKPA PSDRAGDDAA R (b) a protein characterized as M. tuberculosis
antigen 85C; and (c) a glycoprotein characterized as M.
tuberculosis ***antigen*** MPT32.

39. The kit of claim 34, that includes an early ***antigen***
selected from the group consisting of: (a) an 88 kDa M. tuberculosis
protein having the following properties: (1) present in M. tuberculosis
culture filtrate; (2) pI of about 5.2; (3) an amino acid sequence SEQ ID
NO:106.

MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KVVADLTPQN
QALLNARDEL

QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT
TTAGPQLVVP

VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAEKGPTY NKVRGDKVIA
YARKFLDDSV

PLSSGSFGDA TGFTVQDGQL VVALPKSTG LANPGQFAGY TGAAESPTSV
LLINHGLHIE

ILIDPESQVG TTDRAGVKDV ILESATTIM DFEDSVAADV AADKVLGYRN
WLGLNKGDLA

AAVDKDGTAFLRVLNRDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTNDAL
VDTDGSEVFE

GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC
ELFSRVEDVL

GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRVVFINTGF LDRTGDEIHT
SMEAGPMVRK

GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI
AQPRAGASTA

WVPSPTAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA
PDEIRĒEVDN

NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH
GVITSADVRA

SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT
EPILHRRRRE

FKARAAEKPA PSDRAGDDAA R (b) a protein characterized as M. tuberculosis
antigen 85C; and (c) a glycoprotein characterized as M.
tuberculosis ***antigen*** MPT32.

40. The kit of claim 35 that includes an early ***antigen***
selected from the group consisting of: (a) an 88 kDa M. tuberculosis
protein having the following properties: (1) present in M. tuberculosis
culture filtrate; (2) pI of about 5.2; (3) an amino acid sequence SEQ ID
NO:106:

MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KVVADLTPQN
QALLNARDEL

QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT
TTAGPQLVVP

VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAEKGPTY NKVRGDKVIA
YARKFLDDSV

PLSSGSFGDA TGFTVQDGQL VVALPKSTG LANPGQFAGY TGAAESPTSV
LLINHGLHIE

ILIDPESQVG TTDRAGVKDV ILESAITTIM DFEDSVAADV AADKVLGYRN
WLGLNKGDLA

AAVDKDGTAFLRVLNRDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTMDAI
VDTDGSEVFE

GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC
ELFSRVEDVL

GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRVVFINTGF LDRTGDEIHT
SMEAGPMVRK

GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI
AQPRAGASTA

WVPSPTAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA
PDEIREEVDN

NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH
GVITSADVRA

SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT
EPILHRRRRE

FKARAAEKPA PSDRAGDDAA R (b) a protein characterized as M. tuberculosis
antigen 85C; and (c) a glycoprotein characterized as M.
tuberculosis ***antigen*** MPT32.

L5 ANSWER 2 OF 4 USPATFULL

AN 2002:344432 USPATFULL

TI ANTIGEN LIBRARY IMMUNIZATION

IN PUNNONEN, JUHA, PALO ALTO, CA, UNITED STATES

BASS, STEVEN H., HILLSBOROUGH, CA, UNITED STATES

WHALEN, ROBERT GERALD, PARIS, FRANCE

HOWARD, RUSSELL, LOS ALTOS HILLS, CA, UNITED STATES

STEMMER, WILLEM P. C., LOS GATOS, CA, UNITED STATES

PI US 2002198162 A1 20021226

US 6541011 B2 20030401

AI US 1999-247890 A1 19990210 (9)

PRAI US 1998-74294P 19980211 (60)

US 1998-105509P 19981023 (60)

DT Utility

FS APPLICATION

LREP MAXYGEN, INC., 515 GALVESTON DRIVE, RED WOOD CITY, CA, 94063

CLMN Number of Claims: 53

ECL Exemplary Claim: 1

DRWN 21 Drawing Page(s)

LN.CNT 5366

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed to antigen library immunization, which provides methods for obtaining antigens having improved properties for therapeutic and other uses. The methods are useful for obtaining improved antigens that can induce an immune response against pathogens, cancer, and other conditions, as well as antigens that are effective in modulating allergy, inflammatory and autoimmune diseases.

CLM What is claimed is:

1. An recombinant multivalent ***antigenic*** polypeptide that comprises a first ***antigenic*** determinant of a first polypeptide and at least a second ***antigenic*** determinant from a second polypeptide.
2. The multivalent ***antigenic*** polypeptide of claim 1, wherein the polypeptide comprises at least a third ***antigenic*** determinant from a third polypeptide.
3. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first and second polypeptides are selected from the group consisting of cancer ***antigens***, ***antigens*** associated with autoimmunity disorders, ***antigens*** associated with inflammatory conditions, ***antigens*** associated with allergic reactions, and ***antigens*** from infectious agents.
4. The multivalent ***antigenic*** polypeptide of claim 3, wherein the ***antigens*** are from a virus, a parasite, or a bacteria.
5. The multivalent ***antigenic*** polypeptide of claim 4, wherein the ***antigens*** are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus ***complex***, a virus of the tick-borne encephalitis virus ***complex***, a

Dengue virus, a Hanta virus, an HIV, a hepatitis B virus, a hepatitis C virus, and a Herpes simplex virus.

6. The multivalent ***antigenic*** polypeptide of claim 5, wherein the ***antigens*** are envelope proteins.

7. The multivalent ***antigenic*** polypeptide of claim 4, wherein the ***antigens*** are from a bacteria and are selected from the group consisting of a Yersinia V ***antigen***, a Staphylococcus aureus enterotoxin, a Streptococcus pyogenes enterotoxin, a Vibrio cholera toxin, an enterotoxigenic Escherichia coli heat labile enterotoxin, a OspA and a OspC polypeptide from a Borrelia species, an ***Antigen*** 85 polypeptide from a ***Mycobacterium*** species, a VacA and a CagA polypeptide from Helicobacter pylori, and an MSP ***antigen*** from Plasmodium falciparum.

8. The multivalent ***antigenic*** polypeptide of claim 1, wherein the multivalent ***antigenic*** polypeptide exhibits reduced affinity to IgE from a mammal compared to the first or second polypeptides.

9. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first ***antigenic*** determinant and the second ***antigenic*** determinant are from different serotypes of a pathogenic organism.

10. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first ***antigenic*** determinant and the second ***antigenic*** determinant are from different species of pathogenic organism.

11. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are allergens.

12. The multivalent ***antigenic*** polypeptide of claim 11, wherein the allergens are dust mite allergens, grass pollen allergens, birch pollen allergens, ragweed pollen allergens, hazel pollen allergens, cockroach allergens, rice allergens, olive tree pollen allergens, fungal allergens, mustard allergens, and bee venom.

13. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are associated with an inflammatory or autoimmune disease.

14. The multivalent ***antigenic*** polypeptide of claim 13, wherein

the first polypeptide and the second polypeptide are autoantigens associated with a disease selected from the group consisting of multiple sclerosis, scleroderma, systemic sclerosis, systemic lupus erythematosus, hepatic autoimmune disorder, skin autoimmune disorder, insulin-dependent diabetes mellitus, thyroid autoimmune disorder, and rheumatoid arthritis.

15. The multivalent ***antigenic*** polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are cancer ***antigens*** or sperm ***antigens*** .

16. A recombinant ***antigen*** library comprising recombinant nucleic acids that encode ***antigenic*** polypeptides, wherein the library is obtained by recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes a disease-associated ***antigenic*** polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids.

17. The recombinant ***antigen*** library of claim 16, wherein the first and second polypeptides are toxins.

18. A method of obtaining a polynucleotide that encodes a recombinant ***antigen*** having improved ability to induce an immune response to a disease condition, the method comprising: (1) recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes an ***antigenic*** polypeptide that is associated with the disease condition, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library to identify at least one optimized recombinant nucleic acid that encodes an optimized recombinant ***antigenic*** polypeptide that has improved ability to induce an immune response to the disease condition.

19. The method of claim 18, wherein the method further comprises: (3) recombining at least one optimized recombinant nucleic acid with a further form of the nucleic acid, which is the same or different from the first and second forms, to produce a further library of recombinant nucleic acids; (4) screening the further library to identify at least one further optimized recombinant nucleic acid that encodes a polypeptide that has improved ability to induce an immune response to the disease condition; and (5) repeating (3) and (4), as necessary, until the further optimized recombinant nucleic acid encodes a polypeptide that has improved ability to induce an immune response to

the disease condition.

20. The method of claim 18, wherein the disease-associated polypeptides are selected from the group consisting of cancer ***antigens*** ,

antigens associated with autoimmunity disorders,

antigens associated with inflammatory conditions,

antigens associated with allergic reactions, and

antigens associated with infectious agents.

21. The method of claim 18, wherein the disease condition is an infectious disease and the first and second forms of the nucleic acid each encode an ***antigen*** of a different serotype of a pathogenic agent.

22. The method of claim 21, wherein the first and second forms of the nucleic acid are each from a different species of pathogen.

23. The method of claim 21, wherein the screening is accomplished by: introducing into a test animal either: a) the library of recombinant nucleic acids, or b) recombinant polypeptides encoded by the library of recombinant nucleic acids; introducing the pathogenic agent into the test animal; and determining whether the test animal is resistant to challenge by the pathogenic agent.

24. The method of claim 23, wherein the pathogenic agent introduced into the test animal is of a different serotype than that used as a source of the first and second forms of the nucleic acid.

25. The method of claim 23, wherein the library is subdivided into a plurality of pools, each of which pools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

26. The method of claim 25, wherein the pools that include an optimized recombinant nucleic acid are further subdivided into a plurality of subpools, each of which subpools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

27. The method of claim 18, wherein the optimized recombinant nucleic acid encodes a multivalent ***antigenic*** polypeptide and the screening is accomplished by: expressing the library of recombinant nucleic acids in a phage display expression vector such that the

recombinant ***antigen*** is expressed as a fusion protein with a phage polypeptide that is displayed on a phage particle surface; contacting the phage with a first ***antibody*** that is specific for a first serotype of the pathogenic agent and selecting those phage that bind to the first ***antibody*** ; contacting those phage that bind to the first ***antibody*** with a second ***antibody*** that is specific for a second serotype of the pathogenic agent and selecting those phage that bind to the second ***antibody*** ; wherein those phage that bind to the first ***antibody*** and the second ***antibody*** express a multivalent ***antigenic*** polypeptide.

28. The method of claim 27, wherein the screening further comprises contacting those phage that bind to the first and second ***antibodies*** with one or more additional ***antibodies*** , each of which is specific for an additional serotype of the pathogenic agent, and selecting those phage that bind to the respective additional ***antibodies*** .

29. The method of claim 27, wherein the phage display expression vector comprises a suppressible stop codon between the recombinant nucleic acid and the phage polypeptide, whereby expression in a host cell which comprises a corresponding suppressor tRNA results in production of the fusion protein and expression in a host cell which lacks a corresponding suppressor tRNA results in production of the recombinant ***antigen*** not as a fusion protein.

30. The method of claim 18, wherein the optimized recombinant ***antigen*** exhibits an enhanced expression level in a host cell and the screening is accomplished by expression of each recombinant nucleic acid in the host cell and subjecting the host cells to flow cytometry-based cell sorting to obtain those host cells that display the recombinant ***antigen*** on the host cell surface.

31. The method of claim 18, wherein the improved property is selected from the group consisting of: improved immunogenicity; enhanced cross-reactivity against different forms of the disease-associated ***antigenic*** polypeptide; reduced toxicity; improved adjuvant activity in vivo; and improved production of the immunogenic polypeptide.

32. The method of claim 31, wherein the improved property is enhanced cross-reactivity against different forms of the disease-associated polypeptide and the first and second forms of the nucleic acid are from a first and a second form of the disease-associated polypeptide.

33. The method of claim 32, wherein the first and second forms of the disease-associated polypeptide are obtained from at least a first and second species of a pathogenic agent and the optimized recombinant nucleic acid encodes a recombinant polypeptide that induces a protective response against both species of the pathogenic agent.

34. The method of claim 33, wherein the recombinant polypeptide induces a protective response against at least one additional species of the pathogenic agent.

35. The method of claim 33, wherein the pathogenic agent is a toxin.

36. The method of claim 33, wherein the pathogenic agent is a virus or a cell.

37. The method of claim 33, wherein the disease-associated polypeptide is a Yersinia V- ***antigen*** .

38. The method of claim 37, wherein the at least first and second forms of a nucleic acid are obtained from at least a first and second species of Yersinia.

39. The method of claim 38, wherein the Yersinia species are selected from the group consisting of Y. pestis, Y. enterocolitica, and Y. pseudotuberculosis.

40. The method of claim 33, wherein the pathogenic agent is a bacterial toxin.

41. The method of claim 18, wherein the disease condition is cancer and the screening step involves introducing the optimized recombinant nucleic acids into a genetic vaccine vector and testing library members for ability to inhibit proliferation of cancer cells or inducing death of cancer cells.

42. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a tumor specific ***antigen*** .

43. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a molecule which is capable of inhibiting proliferation of cancer cells.

44. The method of claim 18, wherein the disease condition is an

inflammatory response which has an unknown or no ***antigen*** specificity and the screening step involves one or more of the following: a) determining the ability of the genetic vaccine vector to induce cytokine production by PBMC, synovial fluid cells, purified T cells, monocytes/macrophages, dendritic cells, or T cell clones; b) determining the ability of the genetic vaccine vector to induce T cell activation or proliferation; and c) determining the ability of the genetic vaccine vector to induce T cell differentiation to T.sub.H1 or T.sub.H2 cells.

45. The method of claim 18, wherein the disease condition is an autoimmune response.

46. The method of claim 45, wherein the optimized recombinant ***antigenic*** polypeptide shifts the immune response from a T.sub.H1-mediated response to a T.sub.H2-mediated response.

47. The method of claim 18, wherein the disease condition is an allergic immune response.

48. The method of claim 47, wherein the optimized recombinant ***antigenic*** polypeptide shifts the immune response from a T.sub.H2-mediated response to a T.sub.H1-mediated response.

49. The method of claim 47, wherein the optimized recombinant ***antigenic*** polypeptide induces an immune response characterized by predominant IgG and IgM expression and reduced IgE expression.

50. The method of claim 47, wherein the optimized recombinant ***antigenic*** polypeptide is not recognized by pre-existing IgE molecules present in sera of atopic mammals.

51. The method of claim 50, wherein the optimized recombinant ***antigenic*** polypeptide retains T cell epitopes that are involved in modulating a T cell response.

52. A method of obtaining a recombinant viral vector which has an enhanced ability to induce an antiviral response in a cell, the method comprising the steps of: (1) recombining at least first and second forms of a nucleic acid which comprise a viral vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant viral vectors; (2) transfecting the library of recombinant viral vectors into a population of mammalian cells; (3) staining the cells for the presence of Mx protein; and (4) isolating recombinant viral vectors from cells which stain positive for

Mx protein, wherein recombinant viral vectors from positive staining cells exhibit enhanced ability to induce an antiviral response.

53. The method of claim 52, wherein the viral vector comprises an influenza viral genomic nucleic acid.

L5 ANSWER 3 OF 4 USPATFULL

AN 2002:178550 USPATFULL

TI Nucleic acid fragments and polypeptide fragments derived from M. tuberculosis

IN Andersen, Peter, Bronshoj, DENMARK

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PA STATENS SERUM INSTITUT (non-U.S. corporation)

PI US 2002094336 A1 20020718

AI US 2001-791171 A1 20010220 (9)

RLI Division of Ser. No. US 1998-50739, filed on 30 Mar 1998, PENDING

PRAI DK 1997-376 19970402

DK 1997-1277 19971110

US 1997-44624P 19970418 (60)

US 1998-70488P 19980105 (60)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 53

ECL Exemplary Claim: 1

DRWN 6 Drawing Page(s)

LN.CNT 6134

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is based on the identification and characterization of a number of M. tuberculosis derived novel proteins and protein fragments (SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 17-23, 42, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72-86, 88, 90, 92, 94, 141, 143, 145, 147, 149, 151, 153, and 168-171). The invention is directed to the polypeptides and immunologically active fragments thereof, the genes encoding them, immunological compositions such as vaccines and skin test reagents containing the polypeptides. Another part of the invention is based on the surprising discovery that fusions between ESAT-6 and MPT59 are superior immunogens compared to each of the

unfused proteins, respectively.

CLM What is claimed is:

1. A substantially pure polypeptide fragment which a) comprises an amino acid sequence selected from the sequences shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 17-23, 42, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72-86, 88, 90, 92, 94, 141, 143, 145, 147, 149, 151, 153, and 168-171, b) comprises a subsequence of the polypeptide fragment defined in a) which has a length of at least 6 amino acid residues, said subsequence being immunologically equivalent to the polypeptide defined in a) with respect to the ability of evoking a protective immune response against infections with ***mycobacteria*** belonging to the tuberculosis ***complex*** or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with ***antigens*** derived from ***mycobacteria*** belonging to the tuberculosis ***complex***, or c) comprises an amino acid sequence having a sequence identity with the polypeptide defined in a) or the subsequence defined in b) of at least 70% and at the same time being immunologically equivalent to the polypeptide defined in a) with respect to the ability of evoking a protective immune response against infections with ***mycobacteria*** belonging to the tuberculosis ***complex*** or with respect to the ability of eliciting a diagnostically significant immune response indicating previous or ongoing sensitization with ***antigens*** derived from ***mycobacteria*** belonging to the tuberculosis ***complex***, with the proviso that i) the polypeptide fragment is in essentially pure form when consisting of the amino acid sequence 1-96 of SEQ ID NO: 2 or when consisting of the amino acid sequence 87-108 of SEQ ID NO: 4 fused to .beta.-galactosidase, ii) the degree of sequence identity in c) is at least 95% when the polypeptide comprises a homologue of a polypeptide which has the amino acid sequence SEQ ID NO: 12 or a subsequence thereof as defined in b), and iii) the polypeptide fragment contains a threonine residue corresponding to position 213 in SEQ ID NO: 42 when comprising an amino acid sequence of at least 6 amino acids in SEQ ID NO: 42.

2. The polypeptide fragment according to claim 1 in essentially pure form.

3. The polypeptide fragment according to claim 1 or 2, which comprises an epitope for a T-helper cell.

4. The polypeptide fragment according to any of the preceding claims, which has a length of at least 7 amino acid residues, such as at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, and at least 30 amino

acid residues.

5. The polypeptide fragment according to any of the preceding claims, which is free from amino acid residues -30 to -1 in SEQ ID NO: 6 and/or -32 to -1 in SEQ ID NO: 10 and/or -8 to -1 in SEQ ID NO: 12 and/or -32 to -1 in SEQ ID NO: 14 and/or -33 to -1 in SEQ ID NO: 42 and/or -38 to -1 in SEQ ID NO: 52 and/or -33 to -1 in SEQ ID NO: 56 and/or -56 to -1 in SEQ ID NO: 58 and/or -28 to -1 in SEQ ID NO: 151.

6. The polypeptide fragment according to any of the preceding claims which is free from any signal sequence.

7. The polypeptide fragment according to any of the preceding claims which 1) induces a release of IFN- γ from primed memory T-lymphocytes withdrawn from a mouse within 2 weeks of primary infection or within 4 days after the mouse has been rechallenged infected with ***mycobacteria*** belonging to the tuberculosis ***complex***, the induction performed by the addition of the polypeptide to a suspension comprising about 200,000 spleen cells per ml, the addition of the polypeptide resulting in a concentration of 1-4 μ g polypeptide per ml suspension, the release of IFN- γ being assessable by determination of IFN- γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension, and/or 2) induces a release of IFN- γ of at least 300 pg above background level from about 1,000,000 human PBMC (peripheral blood mononuclear cells) per ml isolated from TB patients in the first phase of infection, or from healthy BCG vaccinated donors, or from healthy contacts to TB patients, the induction being performed by the addition of the polypeptide to a suspension comprising the about 1,000,000 PBMC per ml, the addition of the polypeptide resulting in a concentration of 1-4 μ g polypeptide per ml suspension, the release of IFN- γ being assessable by determination of IFN- γ in supernatant harvested 2 days after the addition of the polypeptide to the suspension; and/or 3) induces an IFN- γ release from bovine PBMC derived from animals previously sensitized with ***mycobacteria*** belonging to the tuberculosis ***complex***, said release being at least two times the release observed from bovine PBMC derived from animals not previously sensitized with ***mycobacteria*** belonging to the tuberculosis ***complex***.

8. A polypeptide fragment according to any of the preceding claims, wherein the sequence identity in c) is at least 80%, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, and at least 99.5%.

9. A fusion polypeptide comprising at least one polypeptide fragment according to any of the preceding claims and at least one fusion partner.

10. A fusion polypeptide according to claim 56, wherein the fusion partner is selected from the group consisting of a polypeptide fragment as defined in any of claims 1-8, and an other polypeptide fragment derived from a bacterium belonging to the tuberculosis ***complex***, such as ESAT-6 or at least one T-cell epitope thereof, ***MPB64*** or at least one T-cell epitope thereof, ***MPT64*** or at least one T-cell epitope thereof, and MPB59 or at least one T-cell epitope thereof.

11. A fusion polypeptide fragment which comprises 1) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the M. tuberculosis protein ESAT-6, and a second amino acid sequence including at least one T-cell epitope derived from a M. tuberculosis protein different from ESAT-6 and/or including a stretch of amino acids which protects the first amino acid sequence from in vivo degradation or post-translational processing; or 2) a first amino acid sequence including at least one stretch of amino acids constituting a T-cell epitope derived from the M. tuberculosis protein MPT59, and a second amino acid sequence including at least one T-cell epitope derived from a M. tuberculosis protein different from MPT59 and/or including a stretch of amino acids which protects the first amino acid sequence from in vivo degradation or post-translational processing.

12. A fusion polypeptide fragment according to claim 11, wherein the first amino acid sequence is situated C-terminally to the second amino acid sequence.

13. A fusion polypeptide fragment according to claim 11, wherein the first amino acid sequence is situated N-terminally to the second amino acid sequence.

14. A fusion polypeptide fragment according to any of claims 11-13, wherein the at least one T-cell epitope included in the second amino acid sequence is derived from a M. tuberculosis polypeptide selected from the group consisting of a polypeptide fragment according to any of claims 1-55, DnaK, GroEL, urease, glutamine synthetase, the proline rich ***complex***, L-alanine dehydrogenase, phosphate binding protein, Ag 85 ***complex***, HBHA (heparin binding hemagglutinin), MPT51, ***MPT64***, superoxide dismutase, 19 kDa lipoprotein, .alpha.-crystallin, GroES, MPT59 when the first T-cell epitope is

derived from ESAT-6, and ESAT-6 when the first T-cell epitope is derived from MPT59.

15. A fusion polypeptide fragment according to any of claims 11-14, wherein the first and second T-cell epitopes each have a sequence identity of at least 70% with the natively occurring sequence in the proteins from which they are derived.

16. A fusion polypeptide according to any of claims 11-15, wherein the first and/or second amino acid sequence have a sequence identity of at least 70% with the protein from which they are derived.

17. A fusion polypeptide fragment according to any of claims 11-16, wherein the first amino acid sequence is the amino acid sequence of ESAT-6 or of MPT59 and/or the second amino acid sequence is the amino acid sequence of a *M. tuberculosis* polypeptide selected from the group consisting of a polypeptide fragment according to any of claims 1-8, DnaK, GroEL, urease, glutamine synthetase, the proline rich ***complex***, L-alanine dehydrogenase, phosphate binding protein, Ag 85 ***complex***, HBHA (heparin binding hemagglutinin), MPT51, ***MPT64***, superoxide dismutase, 19 kDa lipoprotein, .alpha.-crystallin, GroES, ESAT-6 when the first amino acid sequence is that of MPT59, and MPT59 when the first amino acid sequence is that of ESAT-6.

18. A fusion polypeptide fragment according to any of claims 11-17, which comprises ESAT-6 fused to MPT59.

19. A fusion polypeptide fragment according to claim 18, wherein no linkers are introduced between the two amino acid sequences.

20. A polypeptide according to any of the preceding claims which is lipidated so as to allow a self-adjuvating effect of the polypeptide.

21. A substantially pure polypeptide according to any of claims 1-20 for use as a pharmaceutical.

22. The use of a substantially pure polypeptide according to any of claims 1-20 in the preparation of a pharmaceutical composition for the diagnosis of or vaccination against tuberculosis caused by ****Mycobacterium**** tuberculosis, ****Mycobacterium**** africanum or ****Mycobacterium**** bovis.

23. A nucleic acid fragment in isolated form which 1) comprises a nucleic acid sequence which encodes a polypeptide as defined in any of

claims 1-20, or comprises a nucleic acid sequence complementary thereto,
2) has a length of at least 10 nucleotides and hybridizes readily under stringent hybridization conditions with a nucleic acid fragment which has a nucleotide sequence selected from

SEQ ID NO: 1 or a sequence complementary thereto,

SEQ ID NO: 3 or a sequence complementary thereto,

SEQ ID NO: 5 or a sequence complementary thereto,

SEQ ID NO: 7 or a sequence complementary thereto,

SEQ ID NO: 9 or a sequence complementary thereto,

SEQ ID NO: 11 or a sequence complementary thereto,

SEQ ID NO: 13 or a sequence complementary thereto,

SEQ ID NO: 15 or a sequence complementary thereto,

SEQ ID NO: 41 or a sequence complementary thereto,

SEQ ID NO: 47 or a sequence complementary thereto,

SEQ ID NO: 49 or a sequence complementary thereto,

SEQ ID NO: 51 or a sequence complementary thereto,

SEQ ID NO: 53 or a sequence complementary thereto,

SEQ ID NO: 55 or a sequence complementary thereto,

SEQ ID NO: 57 or a sequence complementary thereto,

SEQ ID NO: 59 or a sequence complementary thereto,

SEQ ID NO: 61 or a sequence complementary thereto,

SEQ ID NO: 63 or a sequence complementary thereto,

SEQ ID NO: 65 or a sequence complementary thereto,

SEQ ID NO: 67 or a sequence complementary thereto,

SEQ ID NO: 69 or a sequence complementary thereto,
SEQ ID NO: 71 or a sequence complementary thereto,
SEQ ID NO: 87 or a sequence complementary thereto,
SEQ ID NO: 89 or a sequence complementary thereto,
SEQ ID NO: 91 or a sequence complementary thereto,
SEQ ID NO: 93 or a sequence complementary thereto,
SEQ ID NO: 140 or a sequence complementary thereto,
SEQ ID NO: 142 or a sequence complementary thereto,
SEQ ID NO: 144 or a sequence complementary thereto,
SEQ ID NO: 146 or a sequence complementary thereto,
SEQ ID NO: 148 or a sequence complementary thereto,
SEQ ID NO: 150 or a sequence complementary thereto, and
SEQ ID NO: 152 or a sequence complementary thereto,

with the proviso that when the nucleic acid fragment comprises a subsequence of SEQ ID: 41, then the nucleic acid fragment contains an A corresponding to position 781 in SEQ ID NO: 41 and when the nucleic acid fragment comprises a subsequence of a nucleotide sequence exactly complementary to SEQ ID NO: 41, then the nucleic acid fragment comprises a T corresponding to position 781 in SEQ ID NO: 41.

24. A nucleic acid fragment according to claim 23, which is a DNA fragment.

25. A vaccine comprising a nucleic acid fragment according to claim 23 or 24, the vaccine effecting in vivo expression of ***antigen*** by an animal, including a human being, to whom the vaccine has been administered, the amount of expressed ***antigen*** being effective to confer substantially increased resistance to infections with ***mycobacteria*** of the tuberculosis ***complex*** in an animal, including a human being.

26. A nucleic acid fragment according to claim 23 or 24 for use as a

pharmaceutical.

27. The use of a nucleic acid fragment according to claim 23 or 24 in the preparation of a pharmaceutical composition for the diagnosis of or vaccination against tuberculosis caused by ***Mycobacterium*** tuberculosis, ***Mycobacterium*** africanum or ***Mycobacterium*** bovis.

28. An immunologic composition comprising a polypeptide according to any of claims 1-20.

29. An immunologic composition according to claim 28, which further comprises an immunologically and pharmaceutically acceptable carrier, vehicle or adjuvant.

30. An immunologic composition according to claim 29, wherein the carrier is selected from the group consisting of a polymer to which the polypeptide(s) is/are bound by hydrophobic non-covalent interaction, such as a plastic, e.g. polystyrene, a polymer to which the polypeptide(s) is/are covalently bound, such as a polysaccharide, and a polypeptide, e.g. bovine serum albumin, ovalbumin or keyhole limpet hemocyanin; the vehicle is selected from the group consisting of a diluent and a suspending agent; and the adjuvant is selected from the group consisting of dimethyldioctadecylammonium bromide (DDA), Quil A, poly I:C, Freund's incomplete adjuvant, IFN- γ , IL-2, IL-12, monophosphoryl lipid A (MPL), and muramyl dipeptide (MDP).

31. An immunologic composition according to any of claims 28 to 30, comprising at least two different polypeptide fragments, each different polypeptide fragment being a polypeptide according to any of claims 1-67.

32. An immunologic composition according to claim 28, comprising 3-20 different polypeptide fragments, each different polypeptide fragment being according to any of claims 1-20.

33. An immunologic composition according to any of claims 28-32, which is in the form of a vaccine.

34. An immunologic composition according to any of claims 28-32, which is in the form of a skin test reagent.

35. A vaccine for immunizing an animal, including a human being, against tuberculosis caused by ***mycobacteria*** belonging to the tuberculosis ***complex***, comprising as the effective component a

non-pathogenic microorganism, wherein at least one copy of a DNA fragment comprising a DNA sequence encoding a polypeptide according to any of claims 1-20 has been incorporated into the genome of the microorganism in a manner allowing the microorganism to express and optionally secrete the polypeptide.

36. A vaccine according to claim 35, wherein the microorganism is a bacterium.

37. A vaccine according to claim 36, wherein the bacterium is selected from the group consisting of the genera ***Mycobacterium***, Salmonella, Pseudomonas and Eschericia.

38. A vaccine according to claim 37, wherein the microorganism is ***Mycobacterium*** bovis BCG, such as ***Mycobacterium*** bovis BCG strain: Danish 1331.

39. A vaccine according to any of claims 35-38, wherein at least 2 copies of a DNA fragment encoding a polypeptide according to any of claims 1-20 are incorporated into the genome of the microorganism.

40. A vaccine according to claim 39, wherein the number of copies is at least 5.

41. A replicable expression vector which comprises a nucleic acid fragment according to claim 23 or 24.

42. A vector according to claim 41, which is selected from the group consisting of a virus, a bacteriophage, a plasmid, a cosmid, and a microchromosome.

43. A transformed cell harbouring at least one vector according to claim 41 or 42.

44. A transformed cell according to claim 43, which is a bacterium belonging to the tuberculosis ***complex***, such as a M. tuberculosis bovis BCG cell.

45. A transformed cell according to claim 43 or 44, which expresses a polypeptide according to any of claims 1-20.

46. A method for producing a polypeptide according to any of claims 1-20, comprising inserting a nucleic acid fragment according to claim 23 or 24 into a vector which is able to replicate in a host cell, introducing the resulting recombinant vector into the host cell,

culturing the host cell in a culture medium under conditions sufficient to effect expression of the polypeptide, and recovering the polypeptide from the host cell or culture medium; or isolating the polypeptide from a short-term culture filtrate as defined in claim 1; or isolating the polypeptide from whole ***mycobacteria*** of the tuberculosis ***complex*** or from lysates or fractions thereof, e.g. cell wall containing fractions; or synthesizing the polypeptide by solid or liquid phase peptide synthesis.

47. A method for producing an immunologic composition according to any of claims 28-32 comprising preparing, synthesizing or isolating a polypeptide according to any of claims 1-20, and solubilizing or dispersing the polypeptide in a medium for a vaccine, and optionally adding other M. tuberculosis ***antigens*** and/or a carrier, vehicle and/or adjuvant substance, or cultivating a cell according to any of claims 37-45, and transferring the cells to a medium for a vaccine, and optionally adding a carrier, vehicle and/or adjuvant substance.

48. A method of diagnosing tuberculosis caused by ***Mycobacterium*** tuberculosis, ***Mycobacterium*** africanum or ***Mycobacterium*** bovis in an animal, including a human being, comprising intradermally injecting, in the animal, a polypeptide according to any of claims 1-20 or an immunologic composition according to claim 34, a positive skin response at the location of injection being indicative of the animal having tuberculosis, and a negative skin response at the location of injection being indicative of the animal not having tuberculosis.

49. A method for immunising an animal, including a human being, against tuberculosis caused by ***mycobacteria*** belonging to the tuberculosis ***complex***, comprising administering to the animal the polypeptide according to any of claims 1-20, the immunologic composition according to claim 33, or the vaccine according to any of claims 35-40.

50. A method according to claim 49, wherein the polypeptide, immunologic composition, or vaccine is administered by the parenteral (such as intravenous and intraarterially), intraperitoneal, intramuscular, subcutaneous, intradermal, oral, buccal, sublingual, nasal, rectal or transdermal route.

51. A method for diagnosing ongoing or previous sensitization in an animal or a human being with bacteria belonging to the tuberculosis ***complex***, the method comprising providing a blood sample from the animal or human being, and contacting the sample from the animal with

the polypeptide according to any of claims 1-20, a significant release into the extracellular phase of at least one cytokine by mononuclear cells in the blood sample being indicative of the animal being sensitized.

52. A composition for diagnosing tuberculosis in an animal, including a human being, comprising a polypeptide according to any of claims 1-20, or a nucleic acid fragment according to claim 23 or 24, optionally in combination with a means for detection.

53. A monoclonal or polyclonal ***antibody***, which is specifically reacting with a polypeptide according to any of claims 1-20 in an immuno assay, or a specific binding fragment of said ***antibody***.

LS ANSWER 4 OF 4 USPATFULL

AN 2001:86035 USPATFULL

TI Early detection of mycobacterial disease

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PI US 6245331 B1 20010612

AI US 1997-1984 19971231 (9)

PRAI US 1997-34003P 19970102 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P.

LREP Venable, Livnat, Shmuel

CLMN Number of Claims: 28

ECL Exemplary Claim: 1

DRWN 51 Drawing Figure(s); 32 Drawing Page(s)

LN.CNT 4630

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A number of protein and glycoprotein antigens secreted by Mycobacterium tuberculosis (Mt) have been identified as "early" Mt antigens on the basis early antibodies present in subjects infected with Mt prior to the development of detectable clinical disease. These early Mt antigens, in particular an 88 kDa secreted protein having a pI of about 5.2 present in Mt lipoarabinomannan-free culture filtrate, a protein characterized as Mt antigen 85C; a protein characterized as Mt antigen MPT51, a glycoprotein characterized as Mt antigen MPT32; and a 49 kDa protein

having a pI of about 5.1, are useful in immunoassay methods for early, rapid detection of TB in a subject. Also provided are antigenic compositions, kits and methods to useful for detecting an early Mt antigen, an early Mt antibody, and immune complexes thereof. For the first time, a surrogate marker is available for inexpensive screening of individuals at heightened risk for developing TB, in particular HIV-1 infected subjects and other immunocompromised individuals.

CLM What is claimed is:

1. A method for the early detection of active ***mycobacterial*** disease or infection in a subject, comprising assaying a biological fluid sample from a subject having symptoms of active tuberculosis, but before the onset of symptoms identifiable as advanced tuberculosis that is distinguished by (a) smear positivity of sputum for acid fast bacilli, (b) cavitory pulmonary lesions, or both (a) and (b), for the presence of early ***antibodies*** specific for an 88 kDa M. tuberculosis protein which protein has the following properties: (i) present in M. tuberculosis culture filtrate (ii) pI of about 5.2; (iii) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear-positivity of sputum for acid-fast bacilli and cavitory pulmonary lesions, and (iv) non reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis wherein the presence of said early ***antibodies*** specific for said protein is indicative of the presence of said disease or infection.
2. The method of claim 1, which includes assaying said sample for ***antibodies*** specific for one or more additional early ***antigens*** of M. tuberculosis selected from the group consisting of (a) a protein characterized as M. tuberculosis ***antigen*** 85C; (b) a protein characterized as M. tuberculosis ***antigen*** MPT51; and (c) a glycoprotein characterized as M. tuberculosis ***antigen*** MPT32.
3. A method according to claim 1, wherein said subject is a human.
4. A method according to claim 3, wherein said subject is infected with HIV-1 or is at high risk for tuberculosis.
5. The method of claim 1 or 2, comprising, prior to said assaying step, the step of removing from said sample ***antibodies*** specific for cross-reactive epitopes or ***antigens*** of proteins present in M. tuberculosis and in other bacterial genera.
6. The method of claim 1 or 2, wherein said 88 kDa protein is a 741 amino acid protein having the sequence SEQ ID NO: 106: MTDRVSVGNL

RIARVLYDFV NNEALPGTDI DPDSFWAGVD KWADLTPQN QALLNARDEL
 QAQIDKWHRR
 RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT TTAGPQLWP
 VLNARFALNA
 ANARWGSLYD ALYGTDVIPE TDGAEKGPTY NKVRGDKVIA YARKFLDDSV
 PLSSGSFGDA
 TGFTVQDGQL WALPDKSTG LANPGQFAGY TGAAESPTSV LLINHGLHIE
 ILIDPESQVG
 TTDRAVKDV ILESAITTIM DFEDSVAVD AADKVLGYRN WLGLNKGDLA
 AAVDKDGTAF
 LRVLNDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTNDI VDTDGSEVFE
 GIMDALFTGL
 IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC ELFSRVEDVL
 GLPQNTMKIG
 IMDEERRTTV NLKACIKAAA DRWFINTGF LDRTGDEIHT SMEAGPMVRK
 GTMKSQPWIL
 AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI AQPRAGASTA
 WVPSPTAATL
 HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA PDEIREEVDN
 NCQSILGYVV
 RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH GVITSADVRA
 SLERMAPLVD
 RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT EPILHRRRRE
 FKARAAEKPA
 PSDRAGDDAA R.

7. A method according to claim 5, wherein said removing is performed by immunoadsorption of said sample with E. coli ***antigens*** .

8. A method for the early detection of active ***mycobacterial***
 disease or infection in a subject, comprising assaying a biological
 fluid sample: from a subject having symptoms of active tuberculosis, but
 before the onset of symptoms identifiable as advanced tuberculosis that
 is distinguished by (a) smear positivity of sputum for acid fast
 bacilli, (b) cavitory pulmonary lesions, or both (a) and (b), for the
 presence of immune ***complexes*** consisting of an 88 kDa M.
 tuberculosis protein ***antigen*** ***complexed*** with an
 antibody specific for said ***antigen*** , which protein has
 the following properties: (i) present in M. tuberculosis culture
 filtrate (ii) pI of about 5.2; (iii) reactive with ***antibodies***
 found in tuberculosis patients who are in a stage of disease prior to
 the onset of smear-positivity of sputum for acid-fast bacilli and
 cavitory pulmonary lesions, and (iv) non reactive with sera from healthy
 control subjects or healthy subjects with latent inactive tuberculosis,
 wherein the presence of said immune ***complexes*** is indicative of

the presence of said disease or infection.

9. A method according to any one of claims 1-8, which further includes a test that detects ***mycobacterial*** bacilli in a sample of sputum or other body fluid of said subject.

10. The method of claim 9 wherein said biological fluid is urine.

11. The method any claims 1, 2, 3, 4, 5, 6, 7 or 8 wherein said biological fluid is urine.

12. The method of any of claims any of claims 1, 2, 3, 4, 5, 6, 7 or 8 comprising, before said assaying step, the step of obtaining said biological fluid sample from said subject.

13. An isolated 88 kDa M. tuberculosis protein that has the following properties: (a) present in M. tuberculosis culture filtrate; (b) an apparent molecular mass of 88 kDa by SDS-polyacrylamide gel electrophoresis; (c) pI of about 5.2; (d) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear-positivity of sputum for acid-fast bacilli and cavitory pulmonary lesions, and (e) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis.

14. The isolated protein of claim 13 having the amino acid sequence SEQ ID NO: 106: MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD

KWADLTPQN

QALLNARDEL QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI
TTSGVD AEIT

TTAGPQLVVP VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAKEGPTY
NKVRGDKVIA

YARKFLDDSV PLSSGSFGDA TGFTVQDGQL WALPDKSTG LANPGQFAGY
TGAAESPTSV

LLINHGLHIE ILIDPESQVG TTDRAGVKDV ILESAITTIM DFEDSVAAVD
AADKVLGYRN

WLGLNKGDLA AAVDKDGTAF LRVLNRDRNY TAPGGGQFTL PGRSLMFVRN
VGHLMTND AI

VDTDGSEVFE GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM
HGPAEVAFTC

ELFSRVEDVL GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRWFINTGF
LDRTGDEIHT

SMEAGPMVRK GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE
LMADMVETKI

AQPRAGASTA WVPSPATAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT

IPLAKELAWA

PDEIRREVDN NCQSILGYWV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS
SQLLANWLRH

GVITSADVRA SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL
SGAQQPNGYT

EPILHRRRRE FKARAAEKPA PSDRAGDDAA R.

15. An ***antigenic*** composition useful for early detection of M. tuberculosis disease or infection comprising the isolated 88 kDa protein of claim 13 or 14.

16. An ***antigenic*** composition according to claim 15, comprising at least one additional early M. tuberculosis ***antigen*** which composition is substantially free of other proteins that are not M. tuberculosis early ***antigens*** with which said early M. tuberculosis ***antigens*** are natively admixed in a culture of M. tuberculosis.

17. A composition according to claim 16, wherein said additional early ***antigen*** is selected from the group consisting of: (a) a protein characterized as M. tuberculosis ***antigen*** 85C; (b) a protein characterized as M. tuberculosis ***antigen*** MPT51; and (c) a glycoprotein characterized as M. tuberculosis ***antigen*** MPT32.

18. A composition according to claim 16 further supplemented with one or more of the following M. tuberculosis ***antigenic*** proteins having an approximate molecular weight as indicated: (i) a 28 kDa protein corresponding to the spot identified as Ref. No. 77 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (ii) a 29/30 kDa protein corresponding to the spot identified as Ref. No. 69 or 59 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (iii) a 31 kDa protein corresponding to the spot identified as Ref. No. 103 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (iv) a 35 kDa protein corresponding to the spot identified as Ref. No. 66 in FIG. 15A-F, FIG. 18, Table 9 or Table 11 and reacting with monoclonal ***antibody*** IT-23; (v) a 42 kDa protein corresponding to the spot identified as Ref. No. 68 or 80 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (vi) a 48 kDa protein corresponding to the spot identified as Ref. No. 24 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; and (vii) a 104 kDa protein corresponding to the spot identified as Ref. No. 111 in FIG. 15A-F, FIG. 18, Table 9 or Table 11, which spots are obtained by 2-dimensional electrophoretic separation of M. tuberculosis lipoarabinomannan-free culture filtrate proteins as follows: (A) incubating 3 hours at 20.degree. C. in 9M urea, 2% Nonidet P-40, 5% .beta.-mercaptoethanol, and 5% ampholytes at pH 3-10; (B) isoelectric focusing on 6% polyacrylamide isoelectric focusing tube gel

of 1.5mm.times.6.5cm, said gel containing 5% ampholytes in a 1:4 ratio of pH 3-10 ampholytes to pH 4-6.5 ampholytes for 3 hours at 1 kV using 10 mM H.sub.3 PO.sub.4 as catholyte and 20 mM NaOH as anolyte, to obtain a focused gel; (C) subjecting the focused gel to SDS PAGE in the second dimension by placement on a preparative SDS-polyacrylamide gel of 7.5.times.10 cm.times.1.5 mm containing a 6% stack over a 15% resolving gel and electrophoresing at 20 mA per gel for 0.3 hours followed by 30 mA per gel for 1.8 hours.

19. A composition according to claim 16 wherein said 88 kDa M. tuberculosis protein or any one of said additional early M. tuberculosis ***antigens*** is a recombinant protein or glycoprotein.

20. A kit useful for early detection of M. tuberculosis disease comprising: (a) an ***antigenic*** composition comprising an 88 kDa M. tuberculosis protein present in M. tuberculosis culture filtrate having a pI of about 5.2; which is characterized as being (i) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of (1) smear positivity of sputum for acid fast bacilli, (2) cavitary pulmonary lesions, or both (1) and (2), and (ii) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, in combination with (b) reagents necessary for detection of ***antibodies*** which bind to said M. tuberculosis protein.

21. A kit useful for early detection of M. tuberculosis disease comprising: (a) an ***antigenic*** composition consisting essentially of two or more early M. tuberculosis ***antigens*** each of which is characterized as being (i) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of (1) smear positivity of sputum for acid fast bacilli, (2) cavitary pulmonary lesions, or both (1) and (2), and (ii) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, which ***antigenic*** composition includes an 88 kDa M. tuberculosis protein present in M. tuberculosis culture filtrate having a pI of about 5.2; in combination with (b) reagents necessary for detection of ***antibodies*** which bind to said early M. tuberculosis ***antigens***.

22. A kit according to claim 21, wherein at least one of said early M. tuberculosis ***antigens*** is a recombinant protein or glycoprotein.

23. A kit according to claim 21 which further comprises at least one monoclonal ***antibody*** specific for an epitope of one of said

early M. tuberculosis ***antigens*** .

24. A kit useful for early detection of M. tuberculosis disease, comprising: (a) an ***antigenic*** composition that includes: (i) an isolated 88 kDa early M. tuberculosis ***antigen*** which is a protein having the following properties: (1) present in M. tuberculosis culture filtrate; (2) pI of about 5.2; (3) reactive with ***antibodies*** found in tuberculosis patients who are in a stage of disease prior to the onset of smear positivity of sputum for acid fast bacilli, cavitary pulmonary lesions, or both, and (4) non-reactive with sera from healthy control subjects or healthy subjects with latent inactive tuberculosis, (ii) supplemented with one or more isolated early M. tuberculosis ***antigenic*** proteins of a second set, characterized as in (3) and (4) above, obtainable from 14 day cultures of M. tuberculosis strain H37Rv grown in glycerol alanine salts medium, and selected from the group consisting of: (1) a 28 kDa protein having a pI of about 5.1, corresponding to the spot identified as Ref. No. 77 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (2) a 29/30 kDa protein having pI of about 5.1, and corresponding to a spot identified as Ref. No. 69 or 59 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (3) a 31 kDa protein having a pI of about 5.1 and an N-terminal amino acid sequence FSRPGLPVEYLQVPSP (SEQ ID NO: 95), and corresponding to a spot identified as Ref. No. 103 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (4) a 35 kDa protein having a pI of about 5.1 and an N-terminal amino acid sequence CGSKPPSPET (SEQ ID NO: 87), and corresponding to a spot identified as Ref. No. 66 in FIG. 15A-F, FIG. 18, Table 9 or Table 11 and reacting with monoclonal ***antibody*** IT-23; (5) a 42 kDa protein having a pI of about 5.1, and corresponding to a spot identified as Ref No. 68 or 80 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; (6) a 48 kDa protein having a pI of about 4.8, and corresponding to a spot identified as Ref. No. 24 in FIG. 15A-F, FIG. 18, Table 9 or Table 11; and (7) a 104 kDa protein having a pI of about 5.1, and corresponding to a spot identified as Ref. No. 111 in FIG. 15A-F, FIG. 18, Table 9 or Table 11, which spots are obtained by 2-dimensional electrophoretic separation of M. tuberculosis lipoarabinomannan-free culture filtrate proteins as follows: (A) incubating 3 hours at 20.degree. C. in 9M urea, 2% Nonidet P-40, 5% .beta.-mer-captoethanol, and 5% ampholytes at pH 3-10; (B) isoelectric focusing on 6% polyacrylamide isoelectric focusing tube gel of 1.5 mm.times.6.5 cm, said gel containing 5% ampholytes in a 1:4 ratio of pH 3-10 ampholytes to pH 4-6.5 ampholytes for 3 hours at 1 kV using 10 mM H.sub.3 PO.sub.4 as catholyte and 20 mM NaOH as anolyte, to obtain a focused gel; (C) subjecting the focused gel to SDS PAGE in the second dimension by placement on a preparative SDS-polyacrylamide gel of 7.5.times.10 cm.times.1.5 mm containing a 6% stack over a 15% resolving gel and electrophoresing at 20 mA per gel for 0.3 hours

followed by 30 mA per gel for 1.8 hours. said ***antigenic*** composition in combination with: (b) reagents necessary for detection of ***antibodies*** which bind to said early M. tuberculosis ***antigens*** .

25. The kit of claim 24 wherein said ***antigen*** of said second set is the 29/30 kDa protein.

26. A kit according to claim 20, 21, 22, 23 or 24, that includes an early ***antigen*** selected from the group consisting of: (a) a protein characterized as M. tuberculosis ***antigen*** 85C; (b) a protein characterized as M. tuberculosis ***antigen*** MPT51; and (c) a glycoprotein characterized as M. tuberculosis ***antigen*** MPT32.

27. The kit of claim 26 wherein said 88 kDa protein is a 741 amino acid protein having the sequence SEQ ID NO: 106: MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KWADLTPQN QALLNARDEL QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT TTAGPQLWP VLNARFALNA ANARWGSLYD ALYGTDIVPE TDGAKEGPTY NKVRGDKVIA YARKFLDDSV PLSSGSFGDA TGFTVQDGQL WALPDKSTG LANPGQFAGY TGAAESPTSV LLINHGLHIE ILIDPESQVG TTDRAVKDV ILESAITTIM DFEDSVAADV AADKVLGYRN WLGLNKGDLA AAVDKDGTAF LRVLNDRNY TAPGGGQFTL PGRSLMFVRN VGHLMTNDI VDTDGSEVFE GIMDALFTGL IAIHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC ELFSRVEDVL GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRWFINTGF LDRTGDEIHT SMEAGPMVRK GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI AQPRAGASTA WVPSPATAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA PDEIREVDN NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH GVITSADVRA SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT EPILHRRRRE FKARAAEKPA PSDRAGDDAA R.

28. The kit of claim 20, 21, 22, 23 or 24, wherein said 88 kDa protein is a 741 amino acid protein having the sequence SEQ ID NO: 106: MTDRVSVGNL RIARVLYDFV NNEALPGTDI DPDSFWAGVD KWADLTPQN

QALLNARDEL

QAQIDKWHRR RVIEPIDMDA YRQFLTEIGY LLPEPDDFTI TTSGVDAEIT
TTAGPQLWP

VLNARFALNA ANARWGSLYD ALYGTDVIPE TDGAKEGPTY NKVRGDKVIA
YARKFLDDSV

PLSSGSFGDA TGFTVQDGQL VVALPKSTG LANPGQFAGY TGAAESPTSV
LLINHGLHIE

ILIDPESQVG TTDRAKVVDV ILESATTIM DFEDSVAADV AADKVLGYRN
WLGLNKGDLA

AAVDKDGTAFLRVLNRDRNY TAPGGGQFTL PGRSLMFVRN VGHLMNTDAI
VDTDGSEVFE

GIMDALFTGL IAHGLKASD VNGPLINSRT GSIYIVKPKM HGPAEVAFTC
ELFSRVEDVL

GLPQNTMKIG IMDEERRTTV NLKACIKAAA DRWFINTGF LDRTGDEIHT
SMEAGPMVRK

GTMKSQPWIL AYEDHNVDAG LAAGFSGRAQ VGKGMWTMTE LMADMVETKI
AQPRAGASTA

WVPSPTAATL HALHYHQVDV AAVQQGLAGK RRATIEQLLT IPLAKELAWA
PDEIREEVDN

NCQSILGYVV RWVDQGVGCS KVPDIHDVAL MEDRATLRIS SQLLANWLRH
GVITSADVRA

SLERMAPLVD RQNAGDVAYR PMAPNFDDSI AFLAAQELIL SGAQQPNGYT
EPILHRRRRE

FKARAAEKPA PSDRAGDDAA R.